Decreasing the Variability Observed in Urine Analysis

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Abstract. Urine analysis is affected significantly by biological variability. The objective of this study was to study the feasibility of reducing the biological variability of excretion of various analytes in urine, especially albumin in children with diabetes, by mixing small volumes of early morning samples. Twenty-two male children with type 1 diabetes collected early morning aliquots of approximately 10 ml of urine on 3 consecutive days and kept them refrigerated in sealed containers. The urine collection was repeated every 4-6 months in the diabetic children. Ten normal children and 10 normal adults participated as controls. The specimens were analyzed individually and as mixed samples for each subject. Mixing the 3 urine samples before analysis decreased the biological variability of all urine assays (albumin, glucose, creatinine, total protein, potassium). The diabetic children had 3 times higher variability of urine albumin (as a ratio to creatinine) compared to normal children, when the urine samples were collected individually (61% vs 19%, respectively). The variability in the diabetic children decreased when the 3 specimens were analyzed as a single sample after mixing, especially when urine albumin was expressed as a ratio to creatinine. Blood glycated hemoglobin levels correlated better with urine glucose levels when 3 urine samples were mixed before analysis. (received 26 July 2000, accepted 15 September 2000)

Key words: Microalbuminuria, urine glucose, urine collection, diabetes mellitus

Introduction

Urine analysis is affected by the great biological variability observed for most analytes, which exceeds 50% in many instances. However, the analytical or instrumental variability is below 5%. This high biological variability diminishes the clinical significance and utilization of urine analysis in medical practice.

Two approaches are usually adopted to decrease the variability: collecting a large volume of urine over a long period of time (e.g., 24 hr) or collecting random urine samples and reporting the results as a ratio to creatinine. Both approaches have limitations. Among the various tests, two are of special concern in diabetics: urine glucose and albumin (microalbuminuria).

Microalbuminuria (MA), i.e., the urinary excretion of albumin between 30-300 mg/day, is a predictor of diabetic complications in general, and specifically of nephropathy in type 1 diabetes and coronary disease in type 2 diabetes [1]. A major problem with MA is the high biological variability in individual patients, yielding a coefficient of variation (CV) of 30-100% [2-4] or a correlation coefficient (r) of 0.43 between 2 samples 1 yr apart [4]. This variability decreases the usefulness of MA testing when following a particular patient over time. Investigators have tried to decrease this variability by expressing MA results as a ratio to creatinine [5-10], or a timed excretion rate (µg/min) [11,12]. Smulders et al [9] found great variability of MA in adults regardless of the methods of collection or expression.

Many investigators find that overnight urine samples have the least variability [2,9,11,13]. However, the variability of overnight samples for MA still remains relatively high (35-50%) [3,9]. It is impractical to obtain timed samples or 24-hr urine collections in outpatients, especially children. Smulders et al [9] suggested decreasing the variability by using the median of multiple urine collections. This approach is costly, since it entails the analysis of several urine samples.
The previous approaches for urine collection or expression of results have not been widely accepted. A more practical and precise method for collection of urine samples would aid in diagnosing, monitoring, and treating patients with diabetes. We studied the feasibility of reducing the variability of several urine tests by collecting 3 small volumes (~10 ml) of urine on successive mornings and mixing them before analysis. We found the variability of several tests was decreased by this simple approach. The results were calculated as concentrations (eg, mg/L) and as ratios to urine creatinine (eg, mg/g Cr).

Materials and Methods

Subjects. Twenty-two male children with diabetes (mean age 12.9 yr, range 4-17 yr), seen during routine clinic visits, were invited to participate in this study after the consent of their parents was obtained. The study was approved by the Institutional Review Board of Wake Forest University School of Medicine. Ten normal children and 10 normal adults were controls. Early on 3 consecutive mornings prior to a scheduled clinic visit, the child voided ~10 ml of urine into a small plastic-capped bottle (20 ml volume). The child’s parents stored the 3 samples in a refrigerator inside a large plastic-capped container (200 ml volume). The 3 urine samples were brought to the clinic on the day that the last sample was collected. Urine collections were repeated every 4-6 months in diabetic children.

Chemical Analysis. The urine samples were analyzed individually and as mixed samples (equal volumes of 1 ml) for each patient. When the samples arrived in the laboratory, microalbuminuria (MA) was measured by nephelometry (“Array” analyzer, Beckman Instruments, Brea, CA) [14]. The other urine analytes were measured after the samples were stored at -20°C. Urine glucose was measured by the glucose oxidase method with a specific electrode [15] and urine potassium by an ion-specific electrode [15] (Beckman “CX 3” analyzer). Urine creatinine was measured by the Jaffé reaction [15]. A blood specimen collected on the day of the last urine collection was assayed for glycated hemoglobin (glycated Hb) by HPLC using an affinity column (Primus Corporation, Kansas City, MO) [16]. All tests had an analytical variability of <5%.

Statistical Analysis. Microsoft Excel software was used to calculate values for the mean, SD, CV, and correlation coefficient (r) of data sets. Trend analysis was performed according to Conover [17].

Results

None of the urine analytes showed a statistically significant trend with samples from successive days.

The average individual variability of all urine analytes was decreased greatly by mixing the 3 samples and analyzing the composite sample (Table 1). Glucose had an average variability of 106% based on analyses of single samples; the variability was decreased to 20% when 3 samples were mixed. Creatinine had the lowest variability (39%) based on analyses of single samples; it decreased to 2% when 3 samples were mixed.

The mean value for MA was 2 times higher in the diabetic children, compared to normal children (Table 2). The diabetic children had 3 times higher variability of MA in single samples compared to normal children when expressed as mg/L (89% vs. 33%, respectively). The variability of MA in single samples was decreased when expressed as mg/g of creatinine (61% vs 19%), but the difference between the 2 groups remained large.

Analyzing the composite samples, compared to separate samples, reduced the variability of MA in diabetic children, especially when the results were based on creatinine (from 61% to 13%). Reduced variability was also evident when diabetic patients were followed up over a 10-month period, and was also seen in the normal children and adults (Table 2).

The correlation coefficients between the glycated Hb levels in blood and the glucose concentrations in individual urine samples collected on days 1, 2, and 3 were 0.34, 0.63, and 0.64, respectively. The correlation of glycated Hb levels in blood vs urine glucose concentrations was improved (r, 0.71), when 3 urine samples were mixed prior to glucose analysis.

Discussion

We found that mixing an aliquot of 3 successive morning urine samples decreased the variability for all measured analytes, including microalbumin. This simple procedure, even more than the correction based on creatinine excretion, results in lower coefficients of
The procedure for sample collection is practical for both children and adults, and less expensive than analyzing each sample separately. Because of poor correlation with blood glucose concentrations, analysis of urine glucose has been largely discontinued [19-21], except for limited use in managing type 2 diabetics, or for diabetes screening in developing countries [20,21]. Bacterial contamination can affect urine glucose levels. However, urine glucose testing is noninvasive compared to blood glucose testing. In the present study, urine glucose showed greater variability than other analytes. Nonetheless, the glucose levels in the mixed urine samples gave a reasonable correlation (r, 0.71) with the corresponding glycated Hb levels in blood samples.

The coefficients of variation (CV) reported in this study should be viewed as relative indices of individual variability. From a statistical viewpoint, the CV should theoretically decrease by the square root of the number of samples assayed (1.73 for 3 samples). The observed values reflect this theoretical decrease. The authors note that the choice of the optimum number of samples depends on the variability of the test, the desired precision, and the convenience of the patient.

In summary, less variability was observed for MA, glucose, and other analytes when analyses were performed on mixed samples of urine voided on three successive mornings, rather than on single samples.

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References


